

Award Number:  
W81XWH-07-1-0244

**TITLE:**  
The Root Cause of Post-traumatic and Developmental Stress Disorder

**PRINCIPAL INVESTIGATOR:**  
Keith A. Young, PhD

**CONTRACTING ORGANIZATION:**  
Texas A&M University System Health Science Center  
College Station, TX 77854

**REPORT DATE:**  
May 2008

**TYPE OF REPORT:**  
Annual

**PREPARED FOR:** U.S. Army Medical Research and Materiel Command  
Fort Detrick, Maryland 21702-5012

**DISTRIBUTION STATEMENT:** (Check one)

Approved for public release; distribution unlimited

Distribution limited to U.S. Government agencies only;  
report contains proprietary information

The views, opinions and/or findings contained in this report are those of the author(s) and should not be construed as an official Department of the Army position, policy or decision unless so designated by other documentation.

# REPORT DOCUMENTATION PAGE

Form Approved  
OMB No. 0704-0188

Public reporting burden for this collection of information is estimated to average 1 hour per response, including the time for reviewing instructions, searching existing data sources, gathering and maintaining the data needed, and completing and reviewing this collection of information. Send comments regarding this burden estimate or any other aspect of this collection of information, including suggestions for reducing this burden to Department of Defense, Washington Headquarters Services, Directorate for Information Operations and Reports (0704-0188), 1215 Jefferson Davis Highway, Suite 1204, Arlington, VA 22202-4302. Respondents should be aware that notwithstanding any other provision of law, no person shall be subject to any penalty for failing to comply with a collection of information if it does not display a currently valid OMB control number. PLEASE DO NOT RETURN YOUR FORM TO THE ABOVE ADDRESS.

1. REPORT DATE (DD-MM-YYYY) 01/05/2008	2. REPORT TYPE Annual Y1 (07-08)	3. DATES COVERED (From - To) 1 March 2007 - 28 Feb 2008		
4. TITLE AND SUBTITLE The Root Cause of Post-traumatic and Developmental Stress Disorder		5a. CONTRACT NUMBER		
		5b. GRANT NUMBER W81XWH-07-1-0244		
		5c. PROGRAM ELEMENT NUMBER		
6. AUTHOR(S) Keith A. Young, PhD		5d. PROJECT NUMBER		
		5e. TASK NUMBER		
		5f. WORK UNIT NUMBER		
7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)  Texas A&M University System Health Science Center  College Station, Texas 77843		8. PERFORMING ORGANIZATION REPORT NUMBER		
9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)  U.S. Army Medical Research and Materiel Command  Fort Detrick, MD 21702		10. SPONSOR/MONITOR'S ACRONYM(S) MOMRP		
		11. SPONSOR/MONITOR'S REPORT NUMBER(S)		
12. DISTRIBUTION / AVAILABILITY STATEMENT				
13. SUPPLEMENTARY NOTES Approved for public release. Distribution unlimited.				
14. ABSTRACT Our overarching scientific hypothesis holds that serotonergic influences on brain development driven by genetics and early experience induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. We are studying this question using both clinical and basic approaches. Full funding of the main contract and subcontracts was completed in August of 2007, and equipment purchases and setup was completed in the following 3 months. Set-up work to validate the assays and procedures have been largely completed. In the ½ year since full funding, local IRB submissions have been made for projects 1 (PTSD screen in active duty troops), 2 (Fluoxetine treatment of active duty troops) and 5 (MRI and psychophysiological assessments). An additional funding supplement to project 5, which will extend the MRI patient recruitment to include active duty troops at Fort Hood using a newly acquired mobile 3T MRI (provided by the VA) has received funding as part of an additional FY2007-2008 funding supplement. The IRB submission for this supplement to project 5 has also been submitted, and a change in scope has been submitted to MOMRP for review. IRB submission for project 4 (Post-mortem analysis) is in preparation. Approval to proceed with animal work was received in August and at this date, most animals enrolled in Task 1 and 2 have completed the protocol. Initial behavioral analysis indicates that both pre- and post-natal isolation stress alters the behavior of adult animals. Histological and stereological work on the brains from these animals is now in progress to complete these tasks.				
15. SUBJECT TERMS No subject terms provided.				
16. SECURITY CLASSIFICATION OF:  a. REPORT U		17. LIMITATION OF ABSTRACT UU	18. NUMBER OF PAGES 7	19a. NAME OF RESPONSIBLE PERSON USAMRMC
b. ABSTRACT U				19b. TELEPHONE NUMBER (include area code)
c. THIS PAGE U				

Standard Form 298 (Rev. 8-98)  
Prescribed by ANSI Std. Z39-18

## Table of Contents

	<u>Page</u>
<b>Introduction.....</b>	4
Body.....	5
<b>Key Research Accomplishments.....</b>	5
<b>Reportable Outcomes.....</b>	7
<b>Conclusion.....</b>	7
<b>References.....</b>	N/A
<b>Appendices.....</b>	N/A

## INTRODUCTION:

Our overarching scientific hypothesis holds that serotonergic influences on brain development driven by genetics and early experience induce a variation of normal brain anatomy that makes the brain highly susceptible to the effects of severe stress. The goal of Project 1 is to describe the progression of post-deployment stress disorders (PTSD, major depression, suicidality) in active duty troops, and to investigate developmental and environmental factors that influence predisposition to PTSD and depression. In coordination with this effort, we will implement a therapeutic trial of the serotonin reuptake inhibitor fluoxetine, to determine whether it can alter the trajectory of post-deployment PTSD (Project 2: This proposal is funded by CDMRP). Using DNA gathered from Projects 1 and 2 (and 5), Project 3 will investigate genetic factors influencing resiliency and susceptibility to stress disorders and therapeutic response to fluoxetine. Projects 4 and 5 are designed to elucidate basic relationships between genetic variation in the serotonin system, limbic brain anatomy, brain function and behavior. Project 4 will investigate post-mortem anatomy in subjects with major depression, while Project 5 will investigate anatomical and functional brain changes in subjects exposed to varying levels of chronic and traumatic stress. An additional supplement to project 5, which will extend the MRI patient recruitment to include active duty troops at Fort Hood using a newly acquired mobile 3T MRI has received funding as part of an additional FY2007-2008 supplement. Finally, animal models (Project 6) will be used to investigate the development of the brain anatomical stress susceptibility phenotype and to screen for novel agents with potential to treat PTSD and depression. An overarching goal of the Program is integration of data across the projects to compare and contrast the potential for different assessment paradigms (MRI anatomy, fMRI, evoked potentials, startle, genetic profiling) to screen for resiliency and predisposition to post-traumatic and developmental stress disorder stress disorders.

BODY:

## KEY RESEARCH ACCOMPLISHMENTS:

### Administrative:

Full funding of the main contract and subcontracts was completed in August of 2007, and equipment purchases and setup was completed in the following 3 months. Approval to proceed with animal work was received from the Animal Care and Use Review Office in August. In the ½ year since full funding, IRB submissions have been made for projects 1 (PTSD screen in active duty troops), 2 (Fluoxetine treatment of active duty troops) and 5 (MRI and psychophysiological assessments). An additional supplement to project 5, which will extend the MRI patient recruitment to include active duty troops at Fort Hood using a newly acquired mobile 3T MRI has received funding as part of an additional FY2007-2008 supplement. The IRB submission for this supplement to project 5 has also been submitted, and the change in scope has been submitted to MOMRP for review. IRB submission for project 4 (Post-mortem analysis) is in preparation.

### Project Specific:

#### *Project 1 Early progression of post-deployment stress disorders (phase 1: post-deployment)*

- Task 1: Sample 1400 active duty/guard troops < 6 wks post-deployment
  - a. CAPS/depression symptoms
  - b. Stress battery (DRRI, development history, suicidality)
  - c. DNA, cortisol
  - d. RBANS (cognitive screen)

Task 2: Resample/test at ~ 6mo

Task 3: Follow-up contact at 1 year for pathways to mental health care.

*1a Deliverable: Progression of stress disorder in post-deployment troops*

*1b Deliverable: Multiple genetic and psychosocial factors alter the trajectory of military PTSD*

*2a Deliverable: Postdeployment pathways to mental healthcare*

*Progress 03/24/08*

*Awaiting IRB approval*

#### *Project 2 Fluoxetine for post-deployment stress disorder*

Task 1: Fluoxetine/placebo supplementation of standard of care in active duty troops (mo 5- 40)

Task 2: Open label fluoxetine extension

Task 3: Exploratory analysis of factors contributing to fluoxetine response

*No deliverables: funded by PDMRP (awaiting IRB approval)*

#### *Project 3 Serotonin and other genes and biomarkers*

Task 1: Compare biologic factors: susceptible vs. resilient (Project 1) and treatment responsive vs. non-responsive (Project 2)

- a. SERT-ss vs. SERT-sl/ll
- b. Biomarkers

*3a Deliverable: SERT-ss genotype predicts response to traumatic stress*

Task 2: Serotonin and additional genes

*3b Deliverable: Serotonin pathway gene alleles contribute to 5HTTLPR effects on PTSD*

Task 3: Multi-locus analysis

*3c Deliverable: Factors affecting fluoxetine response in treatment of PTSD symptoms*

*Progress 03/24/08*

*New staff to perform the sample storage, DNA extraction and analysis for this Aim have been hired and trained. Storage of DNA on DNA cards has been tested and compared to extraction for blood and brain samples using existing de-identified samples. DNA extracted from blood samples yielded cleaner PCR that was more stable at different DNA storage times, and provided more total DNA for additional genotyping. Because of this improvement, we will use venous blood sampling rather than DNA cards or cheek swabs in the study. A new SERT assay yielding additional SERT variants was tested and compared to the older PCR procedure. The new assay yielded the same S/L calls as the original assay and will be used in the*

experiment, since it provides additional information on genetic variation in the SERT gene. Additional assays for BDNF, COMT, HTR1A and FKBP5 are currently being piloted.

*Project 4* Serotonin and other genetic effects on cellular level brain anatomy.

Task 1. Compare regional volumes and neuronal populations in SERT-ss vs. sl/1l

*4a Deliverable: 5HTTLPR effects on the thalamic/cingulate ratio.*

Task 2. Compare serotonin fiber density in SERT-ss vs. SERT-sl/1l thalamus

*4b Deliverable: 5HTTLPR effects on thalamic and cingulate serotonin fiber density.*

*Progress 03/24/08*

*This project is the final protocol for this project to be submitted for IRB approval. We are preparing the protocol for submission within the next 3 months.*

*Project 5* Serotonin and other genetic effects on regional brain anatomy and function

Task 1: Compare thalamic anatomy and startle/evoked potentials in controls and PTSD with SERT as a cofactor.

*5a Deliverable: Thalamic enlargement and startle in PTSD.*

*5b1 Deliverable: 5HTTLPR short allele is associated with thalamic enlargement and potentiated baseline startle.*

*(NEW aim in 2008) 5b2 : Thalamic enlargement and startle in PTSD in active duty troops*

Task 2: Compare effect of emotional probes on startle/evoked potentials in normal controls and PTSD

*5c Deliverable: Facial fear effects on startle and evoked potentials are potentiated in subjects with the 5HTTLPR short allele.*

*Progress 03/24/08*

*New staff has been hired and training has been completed to perform MRI and psychophysiological data analysis. Equipment to obtain psychophysiological testing has been purchased and set-up is complete. Equipment to perform new aim # 5b2 (MRI assessment in active duty troops) has been designed and purchased. The mobile 3T MRI delivery and pad site at Fort Hood should be completed in 2008 allowing this additional work to be performed in active duty troops. A change in scope to perform this additional work as part of project 5 has been submitted to MOMRP. Using existing, de-identified 3T MRI data from a previous project, we compared thalamic manual outlining procedures to automated segmentation routines (Primarily Freesurfer). The automated procedures were very inaccurate at identifying the ventrolateral borders of the thalamus, and resulted in considerably more variability in thalamic volumes than the manual procedures. Until a better automated procedure is available for thalamic and other subcortical structures, we will continue to manually segment the thalamus and use the segmentation routines to for cortical volume estimation only. Available SERT genotypes for these existing samples provided initial evidence that SERT genetic variations may significantly affect thalamic volumes. Since new software designed specifically for 3T images are expected to be available soon, we will continue to test new products that may allow us to reduce MRI analysis times.*

Project 6 Anatomical and behavioral animal models of developmental stress disorders

Task 1: Develop relevant rodent models

- a. Developmental environmental effects on thalamic/cingulate anatomy, behavior and electrophysiology

a1. Prenatal stress

*6a Deliverable: Prenatal stress reduces the cingulate/thalamic volume ratio and potentiates adult depressive and aggressive behavior.*

a2. Postnatal stress

*6b Deliverable: Early post-natal stress reduces the cingulate/thalamic volume ratio and potentiates adult depressive and aggressive behavior*

- b. Developmental serotonergic effects on thalamic/cingulate anatomy, behavior and electrophysiology

*6c Deliverable: Prenatal elevation of serotonin levels reduces the cingulate/thalamic volume ratio and potentiates adult depressive and aggressive behavior*

Task 2: Use rodent model(s) as screens

- a. Effect of the anatomical brain stress phenotype on ETOH intake

*6d Deliverable: Prenatal stress accentuates ETOH intake and reduces adverse effects of high dose ETOH*

- b. Preclinical testing for PTSD agents

*6e Deliverable: Effect of peritraumatic and adult administration of fluoxetine on development of long-term behavioral patterns in rats*

Progress 03/24/08

New staff has been hired and training to perform animal model development and data analysis. Equipment perform the animal testing has been purchased and set-up is complete. Currently, deliverable 6a and 6b (prenatal and post-natal stress models) are partially completed. Behavioral testing has been completed and the brains are being prepared for histology and stereology. Initial analysis of behavior alone suggests that the early developmental interventions altered behavior. We will complete the histological analysis before proceeding to 6c and Task 2.

REPORTABLE OUTCOMES: None

CONCLUSION: No scientific conclusions have been made at this point in time.

APPENDICES: None.